3 Report of Proceedings,

2 CONFERENCE ON MILK CONCENTRATES, Philadelphia, 1957 1/

March 19 and 20, 1957

Conference was held at the Eastern Utilization Research and Development Division with representatives from industry, the State Agricultural Experiment Stations, universities, milk processors, and the U. S. Department of Agriculture and other Federal agencies participating.

This report summarizes the discussions of the various speakers during the conference. If further details regarding any particular subject are desired, they may be obtained by communicating with the person concerned (see appended list of names and addresses).

Eastern Utilization Research and Development Division
Agricultural Research Service
United States Department of Agriculture
Philadelphia 18, Pennsylvania

PROGRAM

Tuesday, March 19

9:40 a.m.	Introductory Remarks	P. A. Wells, Director Eastern Utilization Research and Development Division
10:00 a.m.	Factors Influencing the Stability of Milk Proteins	C. A. Zittle Eastern Utilization Research and Development Division
10:45 a.m.	Some Effects of the Process of Homogenization on Protein Stability	A. H. Wakeman The Creamery Package Mfg. Co. Chicago, Illinois
11:30 a.m.	Non-Protein Nitrogen Changes in Evaporated Milk During Production and Storage	M. J. Pallansch Eastern Utilization Research and Development Division
12:15 p.m.	LUNCH	
2:00 p.m.	Studies in Atmospheric Oxidation of Fatty Acids	D. Swern Eastern Utilization Research and Development Division
2:45 p.m.	Fatty Acids of Milk	R. W. Riemenschneider Eastern Utilization Research and Development Division
3:30 p.m.	Tour of Laboratory	

Wednesday, March 20

9:00 a.m.	Dried Whole Milk. A New Physical Form	N. C. Aceto Eastern Utilization Research and Development Division
10:00 a.m.	Process Innovations Improve Fresh Milk Concentrate	H. E. Calbert University of Wisconsin Madison, Wisconsin
11:00 a.m.	Frozen Concentrated Milk	Leon Tumerman National Dairy Research Laboratories, Inc. Oakdale, Long Island, New York
12:15 p.m.	LUNCH	
2:00 p.m. to 5:00 p.m.	(a) Taste Evaluation of Stored Evaporated Milk (Report on chemical changes in stored samples to be given by Dr. L. F. Edmondson, Eastern Utilization Research and Development Division.)	
	(b) Demonstration of Dried Whole Milk Product	

INTRODUCTORY REMARKS

by

P. A. Wells, Eastern Utilization Research and Development Division

Dr. Wells welcomed the delegates to the conference. He pointed out that the Experiment Station Directors of the Eastern Region at their meeting last year were in favor of having this year's collaborators' conference devoted to milk concentrates, the same subject discussed at last year's conference. At their recent meeting in New York City the Directors favored either fruits or cheese as the topic for discussion at next year's conference, the final choice being left to us.

Dr. Wells called attention to the recent reorganization of the Agricultural Research Service in which the name of the Eastern Utilization Research Branch was changed to Eastern Utilization Research and Development Division. It was emphasized that the reorganization did not involve any changes in the research program of the Division.

A report of the present conference will be prepared and each person attending will automatically receive a copy. Extra copies will be supplied on request.

FACTORS INFLUENCING THE STABILITY OF THE MILK PROTEINS

by

Charles A. Zittle, Eastern Utilization Research and Development Division

The objective of the present research is to understand the factors influencing the physical stability of milk. To keep the research objective sharply defined certain physical changes in the processing and storage of milk are kept in mind that it would be desirable to explain in physical and chemical terms. When the nature of these physical changes is understood it should be possible to keep them within desirable limits.

The approach to this problem has been through basic research with the purified milk proteins, particularly casein and β-lactoglobulin. The effect of heat, of course, is the item of major interest. This has been studied in the past, but the present studies emphasize the influence of the milk salts such as calcium and sodium ions, phosphate, chloride, and citrate ions. Ions have a strong influence on the stability of proteins, and of these ions calcium is easily the most important. Research studies that have been completed here (1, 2, 3, 4, 5 and 6) illustrate the approach to this problem. Among the current studies are the binding of calcium and phosphate ions to casein, and also the rate at which casein aggregates in the presence of calcium ions. When calcium ions are added to a casein solution at a suitable pH the solution will become milky. This indicates that the casein molecules are clumping together to form large particles which scatter light, thus giving the milky appearance. Casein in this form can readily be sedimented in the ultracentrifuge. This has been done and the amount of calcium that is bound to the aggregated casein determined by analysis. This was done with a 1% casein solution containing 10 mM of calcium chloride per liter. The binding is strongly influenced by pH changing from zero at pH 5 to about 35 moles

calcium per 100,000 g. casein at pH 7.5. Does the change of binding with the pH provide any information on the nature of the binding? The net negative charge on the casein without calcium (obtained from titration data) changes in a parallel manner as does the ionization of the secondary acid group of a substituted phosphoric acid. Since the changes are roughly parallel either total net charge or the charge on the phosphate might determine the binding. If, however, some of the phosphate in casein is doubly bound as has been reported recently there would not be sufficient divalent phosphate to account for the binding. Thus it may be only a coincidence that the amount of phosphate present in casein corresponds to the amount of calcium bound. Specific binding sites probably have a part in binding, but the most important factor in calcium binding appears to be a favorable net negative charge.

Now what is found if in addition to calcium chloride sodium phosphate is also present (5 mM per liter)? Up to pH 6 there is no phosphate bound; neither is there any additional calcium bound. Above pH 6 the binding of phosphate increases rapidly and simultaneously much more calcium is bound. This is the pH region in which the phosphate becomes divalent and apparently this form must be present for complex formation to occur. Formation of the calcium phosphate complex is an old story in the chemistry of milk, but the present results emphasize the importance of pH. When the pH drops, changing divalent to monovalent phosphate, the complex will break up, liberating free calcium. This calcium can combine with casein to push it into the region of greater instability. Also it can be concluded from the importance of the divalent charge on the phosphate that whereas phosphate is a good stabilizer at pH 7 it is a much poorer stabilizer at pH 6.

Will these observations hold true for the natural casein complex in milk? Fortunately, the effect of lowering the pH on the calcium and phosphate composition of the natural complex has been studied by DeKadt and Van Minnen (7). The decreases in binding, with decrease in pH, are about the same as observed with purified casein, but the changes are shifted to the left to lower pH values. A possible explanation for this is that milk contains much more salts than the purified system and in the presence of salts phosphate behaves like a considerably stronger acid. So in a general way the results with the natural complex and the results with casein with added calcium and phosphate are confirmatory.

The rate at which the casein aggregates in the presence of calcium is under study. The apparent absorbance due to the light scattering is measured with a spectrophotometer at a wavelength of 600 millimicrons. This is done at a constant temperature since the reaction is very sensitive to small temperature changes. The measurements to be reported were done with a 1% casein solution at pH 6.3 when 6 mM of calcium chloride per liter is added at 30°C. Aggregation at this temperature proceeds in a regular way. Now if the same solution with this amount of calcium is warmed to 55° it will become too white to read in the instrument, but this is quickly reversible, although not completely, if brought back to 30°. The most interesting result of the experiments shown is that the aggregation is not completely reversible, and the amount of the irreversibility increases as the temperature is raised. It had previously been observed in solubility studies (3) that the reversal of precipitation was not complete. The present method provides a more precise means of study. Characterization of these two aggregates, the

reversible and the irreversible, should give a better understanding of factors contributing to the physical stability of milk.

References

1. Viscosity and flocculation of heated β-lactoglobulin solutions: Effect of calcium concentration and pH. J. Dairy Sci. 39, 514 (1957).

2. Viscosity and opacity of heated β-lactoglobulin solutions: The effect of salts, and oxidizing and reducing reagents. J. Am. Chem. Soc. 79, 126 (1957).

3. Precipitation of calcium caseinate by heat and subsequent reversal. J. Dairy Sci. 39, 1651 (1957).

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SOME EFFECTS OF THE PROCESS OF HOMOGENIZATION ON PROTEIN STABILITY

by
A. H. Wakeman, The Creamery Package Mfg. Company
Chicago, Illinois

There is no process in the dairy industry which is so generally used but so little understood than is homogenization. Homogenization has been used in the evaporated milk industry since prior to 1910 to prevent separation of the fat during storage and increase the viscosity of the finished product. Since that time a great deal of investigation into its effect on the various constituents of milk has been carried out with the main attention focused on fat break-up or dispersion. A brief review of the literature on the "Effects of the Process of Homogenization on Protein Stability" is indicated.

(1) Homogenization decreases the protein stability of milk when fat is present.

(2) Higher fat concentration in the homogenized product decreases protein stability.

(3) Higher homogenization pressures decrease protein stability.

(4) Single stage homogenization valves of the poppet type destabilize more than the multiple stage.

(5) Homogenization at temperatures from 140°F. to 176°F. increase protein stability and give superior butterfat dispersion.

(6) "Destabilization effect is due to the absorption of citrates and phosphates on the fat globules, thus lowering their concentration in the serum proper."

(7) Unhomogenized milk has 2.27% of the milk casein absorbed on the fat globule. Properly homogenized milk has 25.2% of the casein absorbed on the fat globules due to the increased fat area.

(8) Over homogenization can deplete the available membrane material to a degree sufficient to cause oiling off in the finished product.

(9) "The mechanism of homogenization and its effects on the protein are not well understood."

Until 20 years ago it was common practice to homogenize evaporated milk at pressures as high as 4000 pounds pressure using various types of valves and gadgets to produce results. All of the machines were operated at slow speeds, 65-100 R.P.M., and of relatively low capacity, up to 800 G.P.H. These slow speeds and low capacity resulted in a low pumping efficiency as well as wide velocity fluctuations through the homogenization valve on each stroke of the piston. These conditions required higher operating pressures to accomplish satisfactory fat dispersion, which in turn increased the problems related to the proteins. Since that time the sizes of homogenizers have steadily increased in capacity to take care of production demands until now they are available in capacities up to 6000 gallons per hour. These new machines operate at speeds up to 300 R.P.M., resulting in a lesser magnitude of pulsation and, hence, smoother flow through the valves, giving lower operating pressures to accomplish the desired results. One machine has five pistons instead of the normal three, which gives less than a 5% variation in flow through the valve on each stroke. It is an established fact that the pressure required to give satisfactory fat dispersion is the lowest pressure obtained on each stroke of the machine.

For example, if there is a 25% variation in pressure during each stroke, the machine will have to operate at 2250 pounds pressure instead of 1650 pounds pressure to give what is considered proper homogenization. Normally it is considered that all fat particles should be 2 microns or less in a well homogenized sample. In a product run on the machine indicated above at 2250 pounds pressure, the product would pass the microscopic test, but the greatest majority of the fat globules would be much less than 2 microns. Hence, as indicated by the reviewed literature, this product will have much less protein stability than would a product homogenized with more uniform fat globule size such as is produced by the homogenizer with less magnitude of hydraulic pulsations.

During the last 20 years there has also been a great deal of development work on homogenizer valves. All of this work has been directed at producing

a valve to give more uniform homogenization at lower pressures by controlling the flow of the product through the valve. Poppet type valves have a lesser angle with more area. Single service valves give greater day by day uniformity in both the perforated metal and wire types.

The occurrence of cream plug and "spaghetti ring" in homogenized market milk has been observed for a long time. Many people think of it as the same condition but it is not!! Cream plug is normally caused by large fat globules which were not broken down during homogenization or were broken down but reformed and rise the same as they would in unhomogenized milk. "Spaghetti ring" occurs less frequently than cream plug but it has been most difficult to control. This problem, which had lost business and caused a great deal of embarrassment, may be corrected by using a single service valve and retainer having a bell shape conforming to the natural flow of a hydraulic jet impinging at right angles on a flat surface. Many people believe it is associated with a salt balance problem which also affects the proteins.

In summary, it appears from the work which has been done by investigations over the years that any condition which will permit homogenization to give the most uniform fat dispersion with the minimum of fat area should produce a milk of superior protein and fat stability as there will still be an excess of protein complexes and salts to satisfy each fat globule. It is our belief that the increased homogenization effect and protein complex effect can be accounted for on the basis of the longer controlled gradual energy dissipation as the milk passes through, not around, the valve.

NON-PROTEIN-NITROGEN CHANGES IN EVAPORATED MILK DURING PRODUCTION AND STORAGE

by
Michael J. Pallansch
Eastern Utilization Research and Development Division

One objective of research carried out in our Dairy Products Section is to aid in the development of a sterile evaporated milk suitable for reconstitution to an acceptable beverage milk.

The methods of sterilizing evaporated milk now used in the industry produce a milk of remarkable stability but possessing a color and flavor different from fresh whole milk. A high-temperature short-time (HTST) sterilization process, under extensive study, produces an evaporated milk with minimum changes in color and flavor. However, this product is physically unstable during prolonged storage.

In order to gain some knowledge of the mechanisms responsible for the observed instability in HTST milk a number of investigations are being carried out. This report covers information obtained during an investigation of the changes occurring in the non-protein-nitrogen (NPN) fraction of evaporated milk during production and storage. The NPN fraction was studied because small degradative changes occurring in the protein fraction during the production and storage would lead to measurable changes in the NPN fractions.

All milk used during the experiment was obtained from the United States Department of Agriculture herd at Beltsville, Maryland.

The conventional evaporated milks were produced as follows: The milk was adjusted with skim milk to give a fat:solids-not-fat ratio of 1:2.25. It was forewarmed at 203°F. for 10 minutes, then condensed and adjusted to give 26% total solids. One ml. of 6.9% $CaCl_2$ was added to each 155 cc. of condensed milk. The canned samples were placed in a Fort Wayne[1/] batch sterilizer. The samples were given a heat treatment equivalent to 243° for 15 minutes.

The HTST samples were prepared as follows: The milk was adjusted to 3.8% fat by the addition of skim milk. It was forewarmed at 195°F. for 10 minutes, cooled to 130°F., and homogenized at 2500 pounds per square inch. After condensing to 26% total solids, 0.2% Na_2HPO_4 was added to stabilize the milk. The milk was sterilized at 279°F. for 15 seconds and aseptically canned.

Fresh milk samples and samples collected just prior to and just after sterilization were taken for analysis. The remaining samples were stored at 80°F., and specimens for analysis at two week intervals.

The samples were treated with trichloroacetic acid to precipitate the proteins. The supernatant solution obtained upon centrifugation was extracted with four portions of diethyl ether to remove the excess trichloroacetic acid. The resulting aqueous layer was evaporated to a few ml. on a rotary evaporator, made to volume and chromatographed on Dowex 50[1/] ion exchange resin, using the gradient elution technique of Stein and Moore (1).

The chromatographic columns were 150 cm. x 0.9 cm. The columns were water jacketed to permit temperature control by circulation of water from a constant-temperature bath. Dowex 50 resin was used for filling the columns.

The samples placed at the top of the resin bed were absorbed in a narrow band and subsequently eluted with buffers of gradually increasing pH and ionic strength. The effluent fractions were collected in 1 ml. portions using a Technicon[1/] fraction collector.

The effluent fractions were analyzed by a photometric ninhydrin procedure. A 1 cc. portion of ninhydrin reagent was added to each fraction, and after shaking briefly, the samples were heated in a covered boiling water bath for 15 minutes to develop the color. The samples were diluted with 5 ml. of a 50-50 n-propanol-water mixture, cooled to room temperature, shaken to oxidize excess hydrindantin, and read at 570 millimicrons (440 millimicrons for proline and hydroxyproline) using a Coleman Jr. spectrophotometer[1/].

A plot of tube numbers versus optical density gives a series of peaks revealing the presence in milk of a number of ninhydrin-positive compounds. The areas under the peaks were used to determine the concentration of the

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[1/] The mention of trade names in this paper is for identification and implies no endorsement of the product.

components. The optical densities of the blank tubes were subtracted from all tubes to get the net optical density due to the ninhydrin-positive compound. After plotting, a base line was drawn connecting successive flat portions of the curve. The areas bounded by the effluent peaks and the base line are a measure of the amount of each component. A planimeter was used to measure the areas under the peaks. By comparing this area with the area obtained using a known amount of the compound, it is possible to calculate the exact amount of the constituent present.

In practice, only the odd-numbered tubes were analyzed photometrically. The even-numbered tubes corresponding to the individual areas were combined and used for paper chromatographic identification of the component.

For the purpose of identification on paper the solutions were desalted by the procedure of Dreze et al. (2) with the exception that solutions of the basic ninhydrin-positive compounds were desalted on Amberlite IRC-150 resin in the H-cycle and eluted with 5N NH_4OH instead of 4N HCl. The residue remaining after evaporation of the eluting reagents was taken up in 0.05 ml. of water and chromatographed on washed Whatman No. 1 filter paper using the solvent system, methanol-water-pyridine (80-20-4). The spots were identified by comparing their position to that of knowns run simultaneously, by their color with a collidine-containing ninhydrin spray (5% collidine, by volume in 0.2% ninhydrin in 95% ethanol (W:V)), or by specific reagents. Various other supplementary solvent systems were specifically chosen to confirm the identity of many of the peaks. The following ninhydrin-positive compounds were identified by this method: aspartic acid, threonine, serine, proline, glutamic acid, glycine, alanine, valine, lysine, creatinine, arginine, leucine, isoleucine and histidine. Urea was identified directly by Nesslerization after treatment with urease.

Two-dimensional chromatograms of a desalted fraction of protein-free whey obtained by equilibrium dialysis showed the presence of the same amino acids indicated above. The location of these amino acids in the eluate of the Dowex 50[1/] column was also determined by studying the elution patterns of synthetic mixtures of known amino acids.

To date it is known that 30 ninhydrin-positive compounds exist in the NPN fraction of evaporated milk. Identification of all these compounds is being attempted.

The ion exchange patterns of the NPN compounds show that only minor changes occur in the concentration of most of the ninhydrin-positive compounds during the processing and storage of evaporated milk. In both the HTST and conventional evaporated milk, however, there is a marked increase in the ammonia concentration during processing and storage. The ammonia peak of the HTST sterilized evaporated milk was 1.9 times that of the fresh milk from which it was prepared. After 16 weeks' storage the peak had increased to 4.3 times that of the fresh milk. The changes in ammonia concentration are even more marked in the case of the conventionally-produced evaporated milk. The size of the ammonia peak after sterilization is 11.6 times that of the fresh milk from which it was prepared. After 4 weeks' storage at 80°F., the ammonia peak was 11.9 times that of the fresh milk.

It seems improbable that the increase in ammonia is due to a breakdown of other ninhydrin-positive compounds. In the case of HTST milk, the total area

under the peaks of the effluent curve increased from 21.28 to 34.72 planimeter units, while the total area of all non-ammonia peaks changed from 17.20 to 17.22 planimeter units. In the case of the conventional evaporated milk, the total area under all the peaks increased from 19.34 to 50.61 planimeter units as a result of the processing and four weeks' storage. Concomitant with this, the total area of all the non-ammonia peaks decreased from 17.43 to only 15.91 planimeter units.

The increase in ammonia does not result in a pH increase. In fact, the pH was lowest in the samples having the greatest ammount of ammonia. A small pH decrease during storage of evaporated milk has been observed by other workers.

It is conceivable that the ammonia generated during processing and storage comes from the proteins, perhaps from a hydrolysis of their amide groups. A partial hydrolysis of such groups would give rise to free COOH groups which could then form hydrogen bonds with the remaining amide groups. A complete hydrolysis of the amide groups, on the other hand, would leave only free COOH groups having no amide groups with which to form hydrogen bonds. A large amount of such hydrogen bonding might give sufficient agglomeration of the protein molecules to cause precipitation.

After 8 weeks' storage, the HTST evaporated milk shows a considerable amount of protein precipitation, whereas the conventional evaporated milk shows none. At this point, the ammonia generated was 3 times as great for the conventional evaporated milk as for the HTST milk. The far larger increase in ammonia during sterilization of conventional evaporated milk may be the result of an essentially complete hydrolysis of the amide groups. Consequently, there would be no amide groups left to participate in hydrogen bonding. The HTST evaporated milk would still have a considerable percentage of its amide groups intact so it could form hydrogen bonds with the liberated COOH groups. This conceivably could result in protein precipitation.

Bibliography

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STUDIES IN ATMOSPHERIC OXIDATION OF FATTY ACIDS

by
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Atmospheric oxygen is the most universally prevalent, as well as economically important, oxidizing agent for fats and fatty acids. Its action on fats and fatty products may be beneficial or deleterious depending on the conditions and circumstances under which it occurs.

Underlying all investigations of the autoxidation of fats is a desire not only to learn the nature of the products formed but also to understand the

mechanisms involved in their production, since only by control of these mechanisms can the desired products be produced or the undesirable products be avoided. Natural fats are generally too complex to permit drawing far-reaching generalizations concerning the mechanisms involved in autoxidative processes. Hence, much work involving these reactions, especially during the past twenty years, has been carried out with simple substances such as oleic, linoleic, or similar acids and their monoesters, because they can be obtained in pure form. Generalizations made on the basis of results obtained with these simple substances have then been applied to natural fats. In certain cases such generalizations may be valid but as in all cases of reasoning by analogy they may not be entirely justified.

Prior to about 1940 most investigators studied the autoxidation of the fats themselves. This approach not only introduced many additional and complicating reactions but it made the interpretation of analytical results difficult, if not impossible. In addition, it soon became evident that some analytical methods were not reliable when applied to autoxidation mixtures, thus making some of the early conclusions dubious. Furthermore, before 1940 modern instruments, such as ultraviolet and infrared spectrophotometers and the polarograph, and efficient separation methods, such as urea complexing techniques, countercurrent distribution, chromatography, molecular and fractional distillation, and low temperature crystallization, were either not generally available or were incompletely developed.

Modern investigators not only availed themselves of new instruments and separation techniques but they also studied a large number of highly purified model compounds. Many of these were not derived from fats but contained structures known to be present in fats and in autoxidizing systems. The autoxidation process was studied in detail kinetically and greater insight was obtained into the phenomena of catalysis and inhibition.

Any explanation of the process of autoxidation must begin, as in all related oxidation reactions, with an understanding of the nature of the first reaction of oxygen with the double bond system. Until this initial step is known with certainty the subsequent steps of the process must remain more or less speculative. It is for this reason that every theory which has been evolved with regard to the autoxidation of fats has been founded on some concept concerning this initial reaction and upon the chemical nature of the product thus formed.

Much information regarding the mechanism of autoxidation of compounds derived from fats has been obtained by studying the oxidation of simple, monounsaturated, non-fatty compounds, such as cyclohexene, which can be prepared readily in a high state of purity.

To Farmer and his co-workers, however, is due the major credit for developing the hydroperoxide hypothesis of autoxidation, especially in its application to fatty acids, and for substantiating it with convincing experimental evidence. According to Farmer, the autoxidation of practically all unconjugated olefinic compounds proceeds by a chain reaction involving addition of a molecule of oxygen to the carbon atom adjacent to the double bond to form a hydroperoxide having an intact double bond.

The autoxidation of pure monounsaturated fatty acids and esters although autocatalytic is slow at ordinary temperatures (below 60°C.). Ultraviolet radiation, metal catalysts and higher temperatures have been used to speed up the reactions. The rate of oxygen absorption of pure methyl oleate, methyl linoleate, and methyl linolenate has been shown to be about 1:10-12:16-25, at comparable temperatures.

In a logical extension of the earlier work on pure monoolefins of relatively low molecular weight, Farmer and Sutton isolated nearly pure methyl octadecenoate hydroperoxides from methyl oleate autoxidation mixtures which had been oxidized in the presence of ultraviolet to low peroxide levels. Molecular distillation and chromatographic adsorption were used. The hydroperoxide was shown to contain about the theoretical peroxide oxygen content.

The facile formation of hydroperoxides from methyl oleate, and other olefins, had prompted Farmer and other investigators to propose that hydroperoxides are the initial products of autoxidation. Because of the energy required to rupture an α-methylenic C-H bond, and for other reasons, Farmer, Bolland and Gee, and Gunstone and Hilditch almost simultaneously concluded that the initial point of oxidative attack was at the double bond and not at the α-methylene group. It was agreed that double bond attack must occur to only a minor extent, probably in sufficient amount to "trigger" the α-methylenic chain reaction which predominates by far. The rate of oxidation of methylene-interrupted polyunsaturated systems is much higher than that of monoethenoic systems because of the activation of a methylene group by two adjacent double bonds. This double activation results in oxidation rates twenty to forty times as great as in singly unsaturated compounds, making the polyethenoic acids the main source of oxidative rancidity problems.

The current theories of autoxidation of nonconjugated polyunsaturated fatty compounds began to develop when it was observed that conjugation of double bonds occurred in autoxidizing fish oil acids. In the oxidation of ethyl linoleate, the monohydroperoxide which forms was shown by ultraviolet absorption measurements to contain approximately 70% of conjugated diene isomers. This calculation was based on spectral data available at that time, but recently the proportion of conjugated hydroperoxides was shown to be 90%.

The autoxidation of linolenate shows the characteristics of a chain reaction, hydroperoxides form, and double bond migration occurs but the fine details have not yet been developed. At 0°C., 60% of the products are monomeric cis, trans-conjugated diene monohydroperoxides.

Under the mild conditions usually employed in autoxidation, saturated compounds have often been assumed to be inert. It is true that the main problems of autoxidation are associated with unsaturated fatty acids, but it is now known that saturated fatty acids undergo a slow autoxidation, particularly at or above 100°C. to form hydroperoxides which decompose.

It can probably be concluded that during the early stages of the autoxidation process the saturated components of a fat play little or no part in the reactions but that as oxygenated components accumulate, particularly peroxides, the rate of reaction of long-chain saturated compounds begins to be significant. In fact, the oxidation of the saturated components of a fat may play

an important role in the ultimate deterioration of paint films and similar coating materials.

Numerous investigators have studied the secondary products of autoxidation of fatty materials. Recently, it was shown that in the uncatalyzed autoxidation of methyl oleate substantially all of it undergoes single attack by oxygen or peroxides before any significant quantity of multiple attack occurs in the chain.

In the uncatalyzed autoxidation of methyl oleate, polymer formation does not occur until advanced stages. When metal catalysts are present, however, polymers form even in the early stages. The structure of the polymers formed from autoxidizing methyl oleate is not known.

FATTY ACIDS OF MILK

by
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Published results of research and recent work at the Eastern Utilization Research and Development Division on the fatty acid composition of milk fat were discussed. The development of improved fractionation techniques and new spectroscopic methods of analysis in recent years have made possible more comprehensive studies of fatty acid composition. The results of these studies show that milk fat stands at the top of the list for complexity of its fatty acids, containing about 40 different acids compared to 6 to 10 for most edible fats. Collectively, the saturated acids, about 18 in number, constitute from about 60 to 65 percent of the total fatty acids, the unsaturated about 35 to 40 percent.

The saturated acids of milk fat are composed largely of acids with even-number of carbon atoms from butyric (C_4) to lignoceric (C_{24}). Palmitic, stearic, and myristic acids are the principal ones, however, and constitute from 72 to 78 percent of the saturated components - or from 45 to 50 percent of the total acids. The remaining 22 to 28 percent of the saturated acids are composed of a few percent each of butyric, caproic, caprylic, capric, and lauric acids. Trace amounts of branch-chain acids, straight-chain acids with uneven number of carbon atoms, and straight-chain acids with 20, 22, and 24 carbon atoms have also been reported.

The unsaturated fatty acids of milk fat are even more complex than the saturated ones, and their fractionation and determination present a more formidable task for the chemist, because they are present not only in different chain length (from C_{10} to C_{24}) but also in different geometrical configurations. Furthermore, isomers with different position of the double bond in the carbon chain are also present.

Mono-unsaturated acids, decenoic, dodecenoic, and tetradecenoic acids are normally present in milk fat in about 0.1 to 0.4 percent concentration. They have a double bond in the 9,10 position and have only the cis-configuration.

Both cis- and trans- Δ 9-hexadecenoic acids have been reported, together amounting to about 3-5 percent of the total acids. Evidence was obtained for several geometrical and position isomers of octadecenoic acid: cis- and trans- Δ 9 and Δ 11-octadecenoic acids; and trans- Δ 16-octadecenoic acid. The octadecenoic acids represent about 25 percent of the total fatty acids.

There are minor percentages of polyunsaturated acids in milk fat, some with 2, 3, 4 and 5 double bonds. The octadecadienoic acids are the most complex, because they are present in conjugated and non-conjugated isomers as well as geometrical isomers. Evidence for the presence of conjugated cis-trans and trans-trans and non-conjugated cis-cis and possibly cis-trans and trans-trans octadecadienoic acids has been obtained by workers at this laboratory by means of chromatographic fractionation and u.v. and i.r. spectroscopy. This group has also isolated fractions containing at least 80 percent octadecatrienoic acid and eicosatetraenoic acids and a fraction of pure pentaenoic acids. These highly unsaturated acids have essentially the all-cis configuration and were apparently identical with acids normally found in animal depot and tissue fats. Thus the trienoic acid was linolenic, the tetraenoic was arachidonic, and the pentaenoic acids were of the clupandonic type, C_{22} and C_{24} - acids with 5 double bonds and cis-configuration. Collectively, the polyunsaturated acids amount to only from 3 to 5 percent of the total fatty acids.

DRIED WHOLE MILK. A NEW PHYSICAL FORM

by
N. C. Aceto
Eastern Utilization Research and Development Division

Continued surpluses of dairy products have emphasized the importance of expanded utilization research in this field. It is felt that one of the most effective ways to reduce these surpluses is to increase the consumption of whole milk by means of an improved dry whole milk. As a consequence, a broad program of research was initiated by this Division with the ultimate goal of developing a dry whole milk of easy dispersibility, good flavor and storage stability.

As presently conceived, the program will be carried forward in three phases. The first phase is concerned with the preparation of a dry whole milk by batch methods which is initially easy to disperse and of good flavor. The second phase will include a study of the storage stability of the material and factors which influence stability. The third phase will be a translation of the essential processing conditions to a commercially feasible operation. The purpose of this paper is to present a report of the progress of this program which thus far has resulted in whole milk dried in a new physical form and possessing excellent flavor and easy dispersibility even in ice water. The processing variables found to be essential in its preparation and a study of its behavior on storage will be discussed.

Puff-drying methods were investigated because we believe them to be superior to spray or roller drying for the drying of milk. Puff-drying has been

successfully applied to heat sensitive citrus juices and other fruit products in both batch and continuous processing. Therefore, a systematic engineering study was made of the factors responsible for producing a form which would satisfy the requirements of easy dispersibility and good flavor.

In general puff-drying may be defined as the formation of a highly expanded sponge-like structure of dried material from a thin film of liquid concentrate under conditions of high vacuum and low temperature. Thus, a puff-dried material would generally be expected to disperse rapidly because of its inherently large surface area per unit weight, and to possess natural flavor because of the high vacuum, low temperature drying conditions employed. As applied to whole milk, drying at low temperatures would also be expected to prevent protein destabilization, and dehydrating in the absence of oxygen and at low temperatures would greatly reduce atmospheric oxidation.

Puffed structures can be developed by various devices; and for some materials, such as those containing large amounts of sugars, the type of structure may not be of prime importance for rapid dispersibility. With whole milk, however, the structure has been found to markedly influence the dispersibility rate of the dried product. Figure 1 illustrates two possible structures. The puffed form on the left was developed by the evolution of water vapor from a de-aerated concentrate. This is characterized by large, nonuniform bubbles and a preponderance of dense unpuffed intercellular material. Product obtained from such a form disperses very poorly compared to that on the right in Figure 1. The latter was formed by the expansion of entrained gas of low solubility in the concentrated milk, and is characterized by small, uniform bubbles and a minimum of unexpanded intercellular material. To differentiate, the latter is hereafter referred to as a foam.

The mere use of an entrained gas does not insure a form that will disperse readily. In order for entrained gases to be effective in forming a fine grained foam, the concentrate must also be held at a low temperature during the initial drying stage. At temperatures above 55°F., and under the vacuums necessary for foaming, the concentrate will boil before it has expanded sufficiently, and boiling at this point will remove the gas from the unexpanded material. The resulting dried structure will then be similar to a puff formed by water vapor from a deaerated concentrate. Initial experiments using only dissolved gases such as CO_2 indicate that these gases because of their high solubility give structures similar to the water vapor type.

When the absolute pressure within the drier reaches the vapor pressure of the concentrate, vigorous agitation of the structure results from the flashing of water vapor. If the desirable form is to survive it must be rigid enough to withstand this action. Here again low temperatures are desirable because of the increased viscosity. High solids content is another factor which increases the viscosity of the concentrate, and it has been found that materials containing up to about 50% solids can be readily dried as a foam. Beyond this concentration the material forms a gel and, thus, becomes difficult to spread evenly for drying.

Figure 2 shows a block diagram of our present batch process. Fresh pasteurized, homogenized milk was obtained from a local dairy for these studies. It had been homogenized at 145°F. and 2500 psi and then pasteurized at 162°F. for 16 seconds. It contained 3.6 to 3.7% fat and about 8.7% solids-not-fat. The

milk was first concentrated in a high vacuum, falling-film evaporator to 47-50% total solids at a batch temperature of 85 to 100°F. It was then heated to 135°F. and homogenized, first at 4000 psi and then at 500 psi, through a single stage homogenizer of the pulsator type. Immediately prior to homogenization, nitrogen was bubbled through the concentrate from a fritted glass sparger dispersing entrained gas through the material. The concentrated milk was then flowed over stainless steel drying pans to an average depth of 1/16 inch, chilled to 55°F. or below, and dried as a foam in a vacuum shelf drier. The resulting dried mass was crushed lightly through stainless steel screens. A typical drying cycle is illustrated in Figure 3.

It has been found that variations in foam structure are possible. These are probably the consequence of a complex relationship among variables, including viscosity, gas content, temperature, and tray loading of the concentrate; and the rate at which the vacuum is applied in forming the foam. The interrelationship among these variables is not fully understood, so that at present it is not possible to predict the exact structure of the foam which a concentrate will form under a given set of drying conditions. However, we have been able to produce dry whole milk of excellent dispersibility over a wide range of conditions. For example, good structures were obtained using concentrates of 41 to 50% solids, having viscosities of 400 to 2150 centipoises, gas contents of from 22 to 98 ml. per liter of concentrate and drying in a foam representing 35 to 70 fold increase in volume. Almost invariably, however, volume increases of less than about 25 fold resulted in appreciable loss of dispersing properties of the product. Figure 4 shows a comparison of the dispersibility rates of each of 4 different foam structures. They are plots of % of total solids dispersed in 38°F. water vs. stirring time in seconds and were derived using a dispersibility rate test which is a modification of the "ease of dispersion" method of Stone et al.* For each curve six stirring times were used and each stirring time was run in triplicate. When run in this way, the test was shown statistically to have a precision of $\pm$ 1.8%. The curves in Figure 4 indicate that beyond an approximate 30 fold expansion there is a wide range of conditions under which good structures are possible. This is quite significant since the need for a critical set of conditions would make it difficult to achieve consistently good dispersibilities, especially in commercial operations.

The rate of dispersibility can also be affected by comminution of the dried foam. The smaller the particle size the less the dispersibility. Obviously then, bulkier products have better dispersibility. The effect of particle size on dispersibility is shown in Figure 5. Larger particle sizes than 20 mesh result in better dispersibility but at a cost in bulk. Smaller sizes improve the bulk volume but at a cost in dispersibility. Therefore, 20 mesh seemed to us at this stage a good compromise between dispersibility and bulk volume. When compared to instant dry skim milks the 20 mesh product is about 1-1/4 to 2-1/2 times as bulky. It is obvious that further reduction in bulk

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* "The Influence of Lipids on Self-Dispersion and on Ease of Dispersion of Milk Powder." Stone, W. K., et al. Food Technology, 1954, Vol. VIII, No. 8.

consistent with good dispersibility is desirable. Since reduction in particle size is not the answer, other means must be sought.

Additional tests of the physical properties of the foam-dried product showed that there was no crystallization of lactose during processing and that the "free fat" content of the product ranges from 15 to 30% of the total fat when prepared under the usual processing conditions. This "free fat" is somewhat above that of spray dried powders, which usually contain less than 10%, but is considerably under the 90% and above typical of roller dried products. When run through the American Dry Milk Institute solubility index test, the product was shown to contain only trace amounts of undispersed material. Finally, the ultracentrifugal behavior of the serum proteins in milk reconstituted from the foam-dried product was shown to be practically identical to the starting milk, indicating that there is virtually no alteration of the most heat-sensitive of the milk proteins in the new process.

We do not yet fully know the reasons for the unique dispersibility of this product. However, some of the factors which most probably contribute to this property are as follows:

1. Maintenance of the dispersing properties of the non-fat portion, especially the proteins. Lack of alteration of the proteins is evidenced by the ultracentrifugal behavior of the whey proteins and the lack of insolubles in the solubility index test.

2. The fine dispersion of the fat globules and its preservation through the drying step. Microscopic examination of the fat in the concentrate before drying and in the milk reconstituted from the dry product has shown no evidence of coalescing or even clustering. The fat globules remain discrete and are the same size as after homogenization.

3. The geometry of the particles. They are irregular in shape, have one very small dimension without being powdery, and hence have little tendency to cake or ball-up when contacted with water.

As soon as a product having good initial flavor and dispersibility was found, the question uppermost was - how does it keep? Therefore a preliminary study of the effects of time and temperature on the dispersibility and flavor of the foam-dried whole milk was begun. For this test, material containing 29.5% fat and 2-1/2% moisture was crushed to pass through 20 mesh and packaged in cans under an atmosphere containing 98.5% N_2 and 1.5% O_2. The cans were stored at 38, 73 and 100°F. and evaluated for dispersibility and flavor after storage periods of 2 weeks and 1, 2, 4 and 6 months.

Dispersibility rate curves previously described were used to evaluate the effect of storage on the dispersing property. A 13 man screened taste panel evaluated flavor using the ranking technique and the resulting data were analyzed using the analysis of variance procedure. One control sample was a freshly prepared product. The other control was a commercial spray dried whole milk packaged for home use as a beverage. Since this product was not available freshly made, arrangements were made with a local retailer for supply from a new shipment to minimize storage time. Upon receipt it was repackaged under 100% N_2 and stored at -90°F. until used.

The findings as to flavor for specified storage periods were as follows:

1. The flavor had not changed significantly after 3 months at 38°F. or after 1 month at 73°F. It was superior to the commercial spray dried control (stored at -90°F.) after 6 months of storage at 38°F. and equal to the control after 6 months at 73°F.

2. The flavor had deteriorated significantly after 2 weeks at 100°F., but was still equal to the spray dried control after 2 months. After 4 months at 100°F. the foam dried sample was inferior to the spray dried control, which had been stored at -90°F.

3. The taste panel identified the off-flavor which developed during storage as "cooked." Milk flavor experts that were consulted on the other hand identified the early developed off-flavor as predominantly "coconut" or "lactone" and the ultimate flavor developed as stale, cereal, or hay-like. It is significant that no tallowy or rancid flavors were detected.

The fate of dispersibility on storage is a far happier one even though losses were found at the very severe 100°F. condition. Storage at this temperature showed that after 1 month the dispersibility rate in water at 38°F. had decreased considerably. In water at 75°F., however, the material was completely dispersible in 35 seconds after 1 month storage and in 2 minutes after 4 months.

At room temperature storage, however, this unique property remains unchanged for 6 months. The remarkable stability of the product is especially significant since we are told that, in every other case where good dispersibility was attained, it was merely transitory and at times the loss was a matter of hours.

It is obvious from the results of the foregoing storage test that flavor stability is of primary concern. Thus the major effort is currently being directed toward its improvement. The following factors known to affect flavor stability are now under investigation: trace metals contamination, notably copper and iron; free fat; bacteria; enzymes; gas packing; preheating; and product moisture content. We already have indications that moisture contents lower than the 2.5% used in the preliminary storage test significantly improve the keeping quality of the product but work along these lines has not progressed far enough to permit a thorough evaluation. Incidentally, moisture contents well under 1% have been achieved without impairing the flavor and dispersibility of the product. This is another feature of the foam-drying process.

Although we are encouraged by the good dispersibility achieved and its behavior on storage, work will also be done to further improve it, especially in storage at above room temperatures.

Finally, engineering studies are required to translate the more costly batch methods into a commercially practicable continuous operation. Cooperative tests have already been made with the manufacturer of a high vacuum, continuous, solid belt drier of the type now in commercial use for drying coffee and citrus juices. This work showed that, with certain modifications in current design, such a drier should be capable of producing the particular physical form required for good dispersibility. A pilot plant scale drier has been ordered to conduct these translation studies.

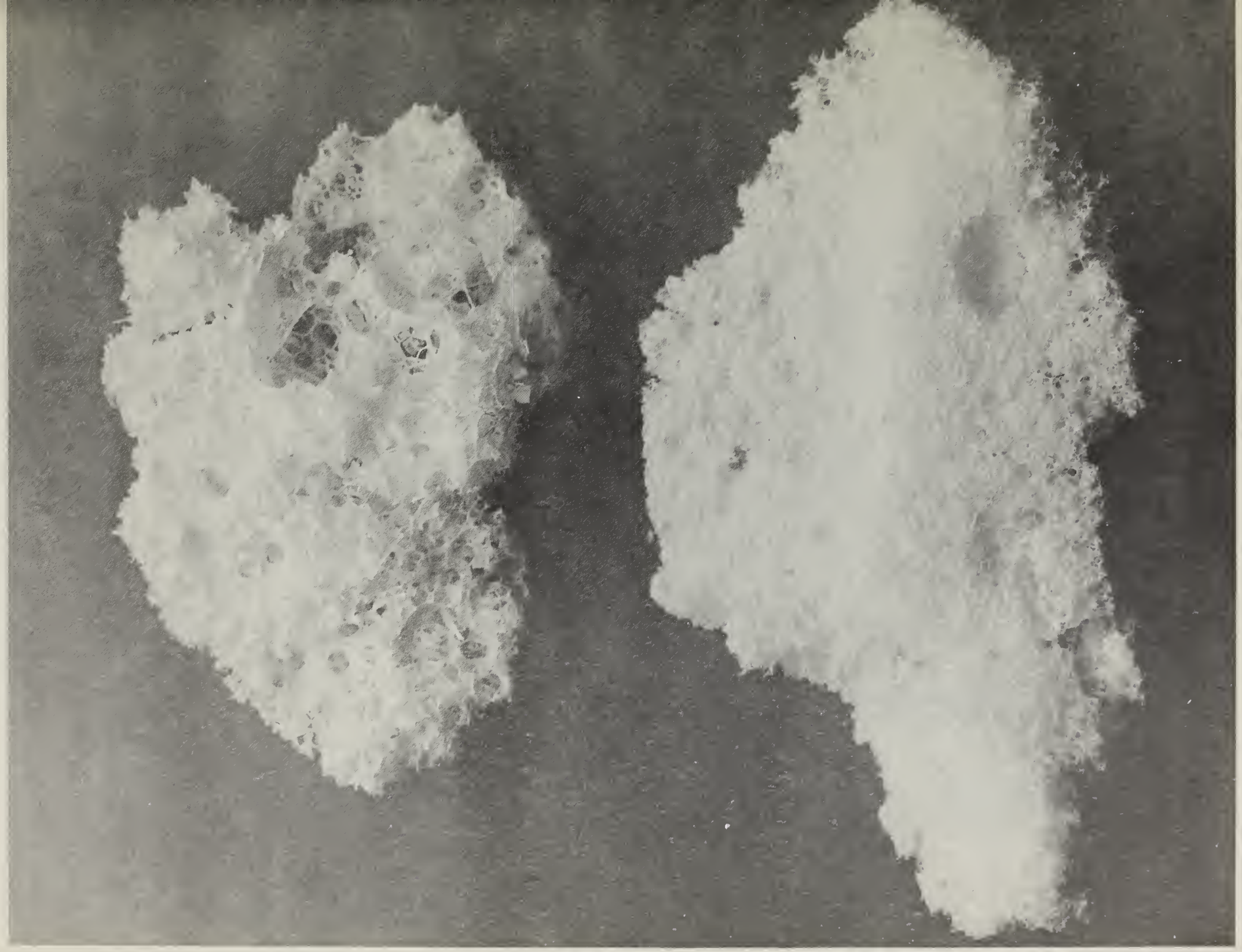
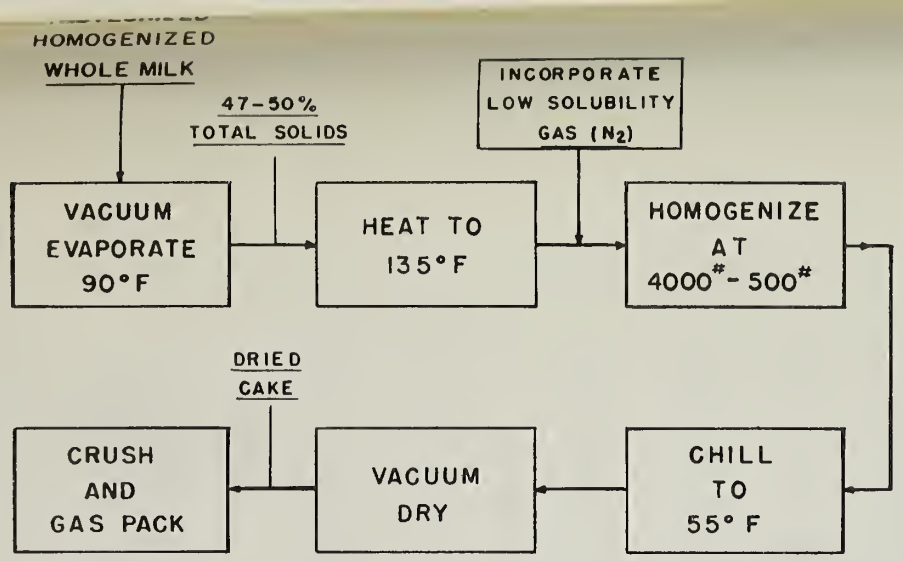


FIG. 1



PROCESS FOR AN IMPROVED DRIED WHOLE MILK

FIG. 2

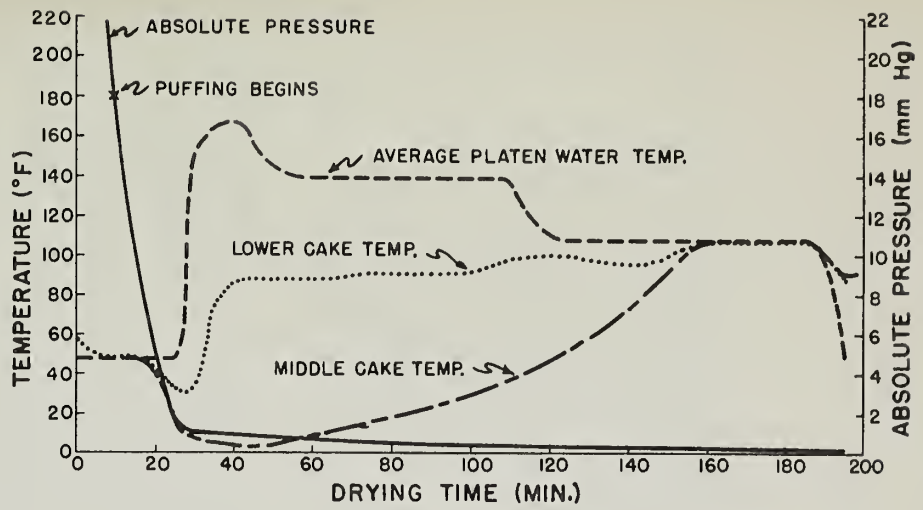


FIG. 3

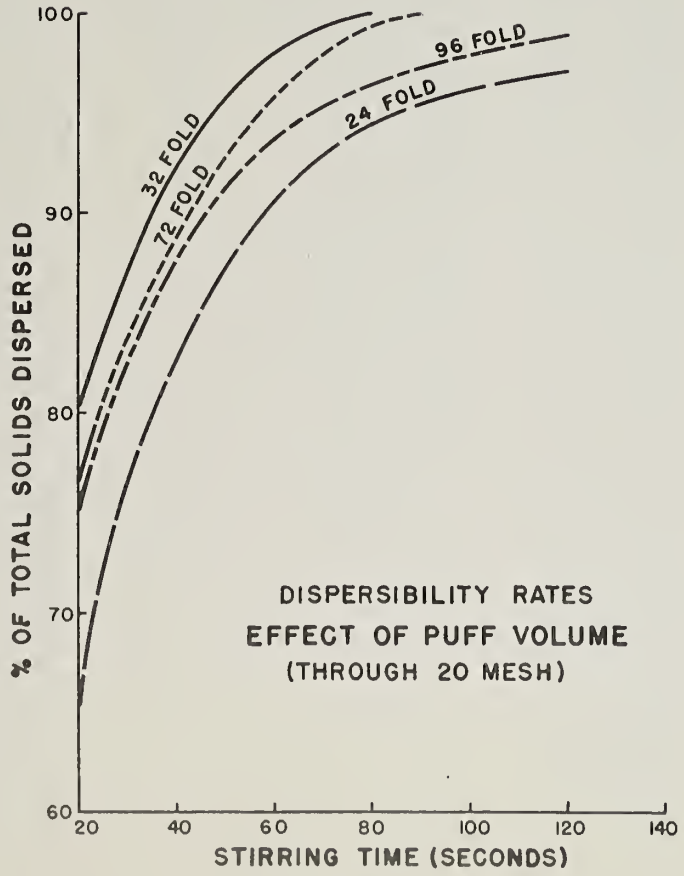


FIG. 4

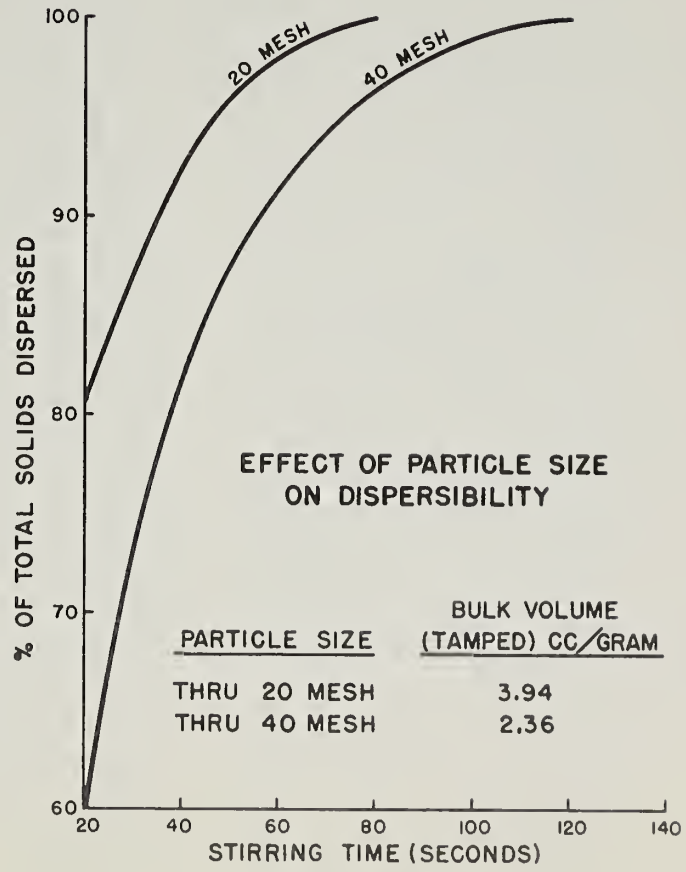


FIG. 5

PROCESS INNOVATIONS IMPROVE FRESH MILK CONCENTRATE

by
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Storage life of several 3-to-1 milk concentrates marketed since World War II did not exceed that of good quality whole milk--about 2 weeks under adequate refrigeration. Through a combination of processing steps using up-to-date equipment, a 3-to-1 concentrate has been developed which possesses the flavor of fresh whole milk and stores well for at least 6 weeks in a normal home refrigerator. Consumer-acceptance surveys conducted among 919 families in Madison and Beloit, Wisconsin, showed that three-fourths of the families liked the concentrate as well as or better than fresh fluid milk as a beverage. When used as a light cream for coffee and for cereals and desserts, reactions were even more favorable.

Early phases of the research showed that when milk was subjected to regular pasteurization before condensing, sterilization of the resulting concentrate at 255-275°F. for 3-4 seconds was inadequate because of a protective effect of the milk solids on the organisms present. In addition, the product had a chalky flavor and gelled early in the storage period. By reversing the procedure--sterilizing before condensing--these objections were overcome. The process developed for producing the superior concentrate is broken down into 9 steps:

1. Start with high quality grade A milk containing ca 3.5% fat and 12% total solids.
2. Heat to 170°F. and homogenize at 2100 psi in single stage unit.
3. Sterilize almost completely by rapid heating to 270°F. and holding for 3 seconds.
4. Evaporate to ca 36.5% solids at 100-110°F.
5. Standardize with sterile water to 36.1% solids.
6. Forewarm to 140-145°F.
7. Heat to 220°F. for 3 seconds.
8. Shock-cool to ca 45°F.
9. Can aseptically in 211 x 214 sanitary type containers with special lacquer liners that have been developed by the can manufacturers. (Fiber containers can be used, but lacquered cans are recommended.)

The improved concentrate is being produced commercially. It is sold through supermarkets for about 2¢ per reconstituted quart less than fresh milk. It is suggested that one factor contributing to public acceptance is the changing shopping habits of the housewife, who commonly shops only once a week. A report of the process has been published by the author and A. M. Swanson in the August 1956 volume of Food Engineering.

FROZEN CONCENTRATED MILK

by
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Although economic considerations have generally restricted the commercial development of frozen concentrated milk, preservation by freezing remains the method best suited to the retention of fresh milk quality over prolonged storage periods. Satisfactory preservation of milk concentrates at temperatures below -15°F. for periods exceeding a year have been recorded. However, the stability of the colloidal caseinate complex in frozen milk is extremely temperature dependent and in the commercially useful temperature range of zero to 15°F., the storage life may not exceed several weeks. At these higher freezing temperatures a gradual coagulation of the protein occurs after a distinct induction period. The amount of coagulated protein then rapidly increases on further frozen storage until substantially all the casein has coagulated. This defect has constituted the major technical barrier to the development of frozen milk, and considerable effort has been expended by academic, government and industrial research laboratories toward resolving this problem. Several process variables were soon recognized as detrimental to the stability of the protein in frozen concentrated milk. These included aging of the concentrate before freezing, the use of forewarming temperatures above 170°F., excessive concentration and high freezing temperatures. The freezing and thawing processes do not of themselves induce any protein coagulation. The coagulum can be repeptized by heat during the early stages of destabilization, but with continued frozen storage progressive irreversibility develops. The coagulum is predominantly calcium caseinate and is substantially free of serum proteins when the milk has been forewarmed below denaturing temperatures. Dialysis, addition of sucrose, ion exchange removal of calcium, or the use of calcium sequestrants under certain conditions stabilize frozen milk, but their commercial application is limited by the serious flavor changes imparted to the product and the variable improvement afforded.

An effective process based on the use of a lactose hydrolyzing enzyme to stabilize frozen milk concentrates was patented by E. G. Stimpson at the National Dairy Research Laboratories. The application of the lactase enzyme and the role of lactose in frozen milk stability will be the subject of this discussion.

The lactose hydrolyzing enzyme is most readily obtained in commercial quantity from yeast cells. Extraction of the enzyme is essential to eliminate those components of the yeast cell that would contribute an objectionable flavor to the treated milk. The hydrolysis of lactose by lactase enzyme is analogous to the inversion of sucrose by invertase. The primary products of the hydrolysis are the monose components, glucose and galactose. A number of oligosaccharides have been identified by chromatographic analysis as additional products of enzymatic hydrolysis. Dried yeast lactase preparations will retain adequate potency over protracted storage periods. The activity of the enzyme used in the production of commercial quantities of stabilized milk is such that one part of enzyme to 40 parts of lactose will ensure 75% hydrolysis in skim milk condensed to 30% total solids. This hydrolysis level is generally attained within 4 hours at 100-110°F. The extent of hydrolysis is suitably measured by a modified Tauber-Kleiner method. On completion of the

enzymatic treatment the condensed skim milk is heated to 155°F. for 15 minutes to inactivate the enzyme, and sufficient cream is added to standardize the product to 35% total solids including 10.5% fat. The untreated concentrate is then standardized with the hydrolyzed product to provide the desired stability. In practice, approximately 10 lbs. of lactase enzyme is sufficient to process 10,000 quarts of milk, whose useful storage life at a freezing temperature of 15°F. will be at least twice that of unhydrolyzed controls. This enzyme requirement is based on a 10% lactose hydrolysis level in the final product.

The role of lactose in frozen milk stability as suggested by current experimental evidence may be summarized as follows:

1. Milk concentrated to approximately 1/3 volume before freezing enters the frozen state with a favorably modified ionic equilibrium and the caseinate complex is relatively stable. The lactose, an essential component in the stability of the casein, mediates against the coagulating action of the salts in some undefined manner. When the protective influence of the lactose is withdrawn by crystallization, casein coagulation proceeds rapidly.

2. Although the total milk solids in the unfrozen phase is independent of starting concentration, the ionic equilibrium may vary sufficiently to influence protein stability. This appears to apply to fluid milk and concentrates at solids levels lower than 3:1. Under these conditions, the protective effect of the lactose is inadequate and the suppression of calcium activity by added citrate or ion exchange removal effectively stabilizes the frozen product.

3. The crystallization of lactose in frozen concentrated milk is initiated by lactose crystal nuclei that are preformed during the process stages at temperatures where the saturated lactose is in a labile state.

4. Enzymatic hydrolysis of a minor portion of the lactose is an effective and economic process for extending the frozen storage life of concentrated milk.

Several mechanisms can be postulated to explain the protective influence of lactose and substitute compounds, such as sucrose, dextrose and maltose, in frozen milk:

1. Many sugars, including lactose form molecular compounds with calcium salts. This reduction of calcium activity would be favored by the high concentration of milk salts and lactose in the frozen milk.

2. The freezing point depression by the solute sugar molecules lowers the effective concentration of salt and protein in the milk at any freezing temperature and would thereby tend to moderate the salting-out effect. However, the Canadian researcher D. Rose observed that the protective influence of different sugars is not equivalent on a molar basis.

3. The high viscosity created in the frozen milk by the sugar component may suppress the diffusion and orientation of caseinate micelles essential to coagulation.

4. The sugars may intervene in the structural organization of the protein so as to intercept sites at which molecular aggregation could occur. Numerous examples of a similar protection afforded proteins by sugars and other polyhydric compounds have been uncovered. Sucrose suppresses the insolubilization of freeze dried lipovitellin, caseinate complex, and other proteins stored at high relative humidities. Inhibition of protein denaturation and complex formation in blood serum proteins by sugars and sugar alcohols have been reported and the clotting of activated fibrinogen can be arrested by a number of polyhydric compounds.

The exact mechanism of caseinate stabilization by sugar in frozen milk remains obscure and further research along these lines is indicated.

LIST OF ATTENDANCE

Name	Organization	Address
Aceto, N. C.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Alstrand, D. V.	American Can Co.	Maywood, Ill.
Angst, C. L.	The Nestle Co., Inc.	White Plains, N.Y.
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Arrigoni, L.	Consolidated Dairy Products Co.	Seattle, Wash.
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Bryan, J. S.	Walker-Gordon Laboratory Co.	Plainsboro, N. J.
Bryant, R. S.	Victor Chemical Works	Chicago, Ill.
Bundus, R. H.	Beatrice Foods Co.	Chicago, Ill.
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Derber, D. W.	United States Steel Corp.	Pittsburgh, Pa.
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Freeman, T. R.	Kentucky Agric. Expt. Sta.	Lexington, Ky.
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Haymes, P. L.	United Milk Products Co.	Cleveland, Ohio
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Kronick, D.	Department of Public Health	Philadelphia, Pa.
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Larson, R. A.	Continental Can Co., Inc.	Chicago, Ill.
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Petersen, F. D.	Industrial Processes Co.	Philadelphia, Pa.
Peterson, R. F.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Plagge, I. F.	Bowey's Inc.	Chicago, Ill.
Porges, N.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Potter, F. E.	Massachusetts Agric. Expt. Sta.	Amherst, Mass.
Ratchford. W. P.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Reeve, R. K.	White House Milk Co., Inc.	Manitowoc, Wisc.
Richards, C. R.	Delaware Agric. Expt. Sta.	Newark, Del.
Riemenschneider,R.W.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Robertson,E.L.(Mrs.)	United States Air Force	Washington, D. C.
Rogers, L. V.	Eastern Util. Res. & Devel. Div.	Washington, D. C.
Sampey, J. J.	Abbotts Dairies, Inc.	Philadelphia, Pa.
Schaffner, R. M.	Libby, McNeill and Libby	Chicago, Ill.
Schoppet, E. F.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Schultz, J. T.	Abbotts Dairies, Inc.	Philadelphia, Pa.
Shiffman, M. A.	Department of Public Health	Philadelphia, Pa.
Silver, S.	Walker-Gordon Laboratory Co.	Plainsboro, N. J.
Sinnamon, H. I.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Snyder, H. L.	United States Steel Corp.	Pittsburgh, Pa.
Steen, C. A.	Walker-Gordon Laboratory Co.	Plainsboro, N. J.
Stone, W. K.	Virginia Agric. Expt. Sta.	Blacksburg, Va.
Swanson, A. M.	University of Wisconsin	Madison, Wisc.
Swern, D.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Tamsma, A.	Eastern Util. Res. & Devel. Div.	Washington, D. C.
Tarassuk, N. P.	University of California	Davis, Calif.
Timasheff, S. N.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Tittsler, R. P.	Eastern Util. Res. & Devel. Div.	Washington, D. C.
Tobler, F. R.	Western Hemisphere Research, Inc.	Marysville, Ohio
Trimble, J. M.	Dean Milk Co.	Rockford, Ill.
Tumerman, L.	National Dairy Research Laboratories	Oakdale,L.I.,N.Y.
Vaurio, V. W.	United States Steel Corp.	Monroeville, Pa.
Wakeman, A. H.	The Creamery Package Mfg. Co.	Fort Atkinson,Wisc.
Walsh, R. M.	Agricultural Marketing Service, USDA	Washington, D. C.
Watt, B. K. (Mrs.)	Agricultural Research Service, USDA	Washington, D. C.
Webb, B. H.	National Dairy Research Laboratories	Oakdale,L.I.,N.Y.
Weil, L.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Weinreich, C. F.	Cherry-Burrell Corp.	Chicago, Ill.
Wells, P. A.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.
Whitnah, C. H.	Kansas State College	Manhattan, Kans.
Williams, A. W.	The Borden Co.	New York, N. Y.
Zittle, C. A.	Eastern Util. Res. & Devel. Div.	Philadelphia, Pa.